Physiol. Res. 51: 17-25, 2002

Mismatch of Local Blood Flow and Oxidative Metabolism in Stunned Myocardium

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Received March 20, 2001
Accepted June 8, 2001

Summary

Myocardial blood flow is spatially heterogeneous, reflecting nonuniform oxygen supply. Also, myocardial oxidative metabolism is spatially heterogeneous. The effects of acute ischemia and reperfusion on the relationship between local myocardial blood flow (LMF) and oxidative metabolism are still unknown. LMF was measured in isolated, blood-perfused rabbit hearts using colored microspheres and oxidation water labeled with $^{18}O_2$ ($H_2{}^{18}O$). Three protocols were performed: $^{18}O_2$-perfusion during normoxia (N; n=7), during early reperfusion (ER; 10 min**,** n=6), and late reperfusion (LR; 40 min, n=6) following 20 min no-flow ischemia. LMF and local $H_2{}^{18}O$ residues were determined within defined myocardial samples (105 ± 15 mg). For interindividual comparison, values were normalized to the mean of the individual experiment and expressed as percentages. LMF ranged from 18 to 193 % (N), 12 to 250 % (ER), and 11 to 180 % (LR). The $H_2{}^{18}O$ tissue residue ranged from 63 to 132 % (N), 73 to 142 % (ER) and 32 to 148 % (LR). The correlation between LMF and local oxidative metabolism during N (r=0.77; n=56) was lost in the postischemic heart during ER and LR. LMF during N and ER were only weakly correlated (r=0.24; n=48), whereas LMF during N and LR correlated well (r=0.87; n=48). It is concluded that the heterogeneous LMF pattern at baseline is maintained in the stunned myocardium whereas that of local oxidative metabolism is not. Apart from the established mechanisms underlying myocardial stunning, a mismatch between local flow and oxidative metabolism might also contribute.

Key words
Myocardial stunning • Local blood flow • Local oxidative metabolism • Perfusion-metabolism mismatch • Heterogeneity

Introduction

Local blood flow within mammalian myocardium is heterogeneously distributed: it varies between 20 % of the mean in low-flow areas and 200 % in high-flow areas (Deussen *et al.* 1996, King *et al.* 1985, Sonntag *et al.* 1996).

The reversible myocardial dysfunction that follows brief periods of ischemia, i.e. myocardial stunning (Heyndrickx *et al.* 1975, Bolli 1990) is more pronounced in areas where the preceding ischemia is more severe (Bolli *et al.* 1989). Whereas stunning appears to be related to the preceding ischemic blood flow, there is no relation to the instantaneous blood flow during reperfusion (Thaulow *et al.* 1989). However, the

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relation of blood flow and metabolism during reperfusion has not yet been studied at the spatial resolution that is necessary for analyzing the above blood flow heterogeneity.

Recently, while measuring both local oxidative metabolism and local myocardial flow, we found a correlation between these two variables in the normoxic, buffer-perfused heart (Schwanke *et al.* 2000). In the present study, we have investigated whether or not this correlation also holds for the blood-perfused myocardium, and whether it is not altered in the stunned myocardium.

Methods

Experiments on 19 isolated hearts from adult New Zealand White rabbits (age 5 - 8 months, weight 3100 – 3800 g) were performed in accordance with the animal welfare regulations of the German authorities that adhere to the guiding principles of the Helsinki Declaration.

The hearts were connected to a modified Langendorff apparatus and perfused with a Krebs-Henseleit solution containing bovine erythrocytes (hct = 30 %; Hb = 10 g/dl), 40 g/l bovine serum albumin and 5000 IU/l heparin. The Krebs-Henseleit composition was as follows (in mM): NaCl (90), $NaHCO_3$ (30), KCl (4), Na_2HPO_4 (1), $MgSO_4$ (0.5), $CaCl_2$ (2.5). In addition to pyruvate (2.2 mM) and glucose (11.1 mM), the perfusate contained free fatty acids bound to albumin (1.4 mM).

This perfusate – it will be called 'blood' throughout this paper – was equilibrated with 72 % N_2, 22 % O_2, 6 % CO_2 or alternatively with 72 % N_2, 22 % $^{18}O_2$, 6 % CO_2 using an infant oxygenator (D705 Midiflow; Dideco; Mirandola, IT) and its temperature was maintained at 38 °C.

Using a servo-controlled roller pump (WM 505, Watson Marlow, Falmouth, GB), coronary arterial pressure (CAP; Statham P23, Gould Nicolet, Erlensee, DE) was adjusted to 80 mm Hg. Coronary blood flow (T206, Transonic Systems Inc, Ithaka/NY, US) and the difference in arterio-venous oxygen content (A-VOX Systems, Inc; San Antonio, TX, US) were measured in the perfusion circuit that contained a blood filter (40 µm; AF-1040 GOLD, Baxter Bentley; Irvine, CA, US) (Fig. 1 left).

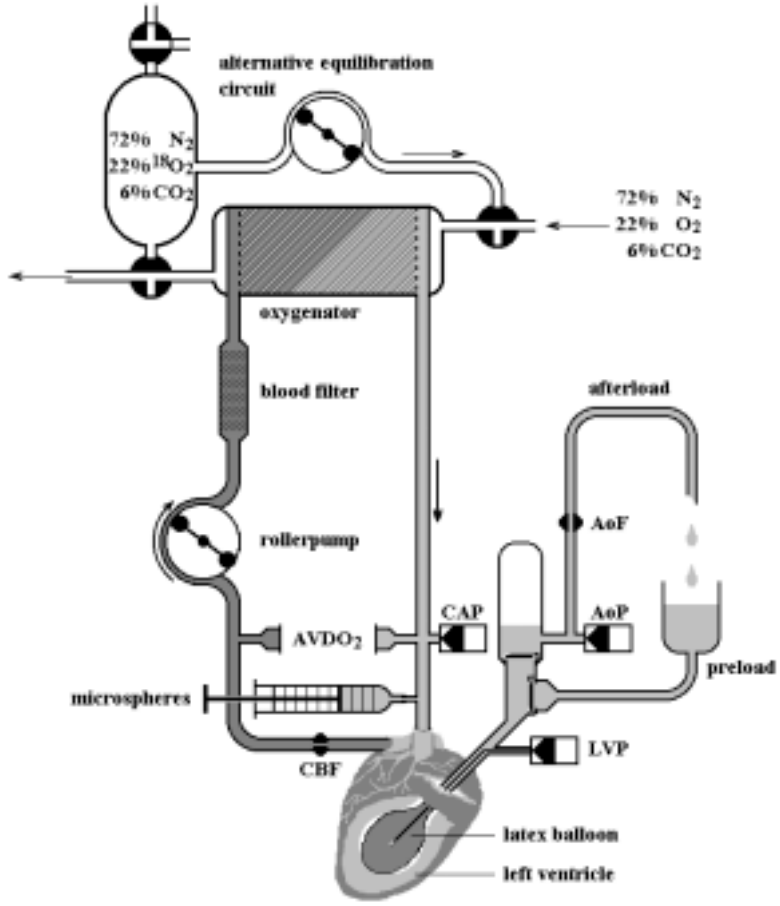

Fig. 1. *Modified Langendorff ap-paratus for blood perfusion of isolated rabbit hearts. In the systemic circuit (right), left ventri-cular pressure (LVP), aortic pressure (AoP), and aortic flow (AoF) and in the perfusion circuit (left) coronary arterial pressure (CAP), global myocardial blood flow (CBF), and the difference in coro-nary arterio-venous oxygen content ($AVDO_2$) were measured. An op-tional gas circuit containing 22 % $^{18}O_2$ permitted labeling of cardiac oxidation water (top).*

A latex balloon (HSE, #12-14, H. Sachs Elektronik, March-Hugstetten, DE) was inserted into the left ventricle *via* the mitral valve. The balloon was connected to an artificial systemic circuit that was separated from the perfusion circuit. To assess ventricular function, left ventricular pressure (TC-500, Millar Instruments, Houston/TX, US) and cardiac output (T206, Transonic Systems Inc, Ithaka/NY, US) were measured in the systemic circuit (Fig. 1 right).

Blue and red microspheres (Kowallik *et al.* 1991; ∅ = 15 µm) were used to measure local myocardial blood flow (LMF). After sonication (RK156, Sonorex, Bandelin electronic, Berlin, DE) and 1 min vigorous shaking (VF1 Vibrofix, IKA-Labortechnik, Staufen i.B., DE), the spheres were injected into the perfusion line directly above the aortic root.

Experimental protocol

After excision and transfer into the Langendorff apparatus, hearts were allowed to stabilize for 15 min. In the following 15-min period, hemodynamic baseline values were assessed. At the end of each protocol, the perfusion was abruptly terminated, and the hearts were immersed into liquid nitrogen. Three different protocols were followed:

Normoxia

Following baseline measurements, normoxic hearts (n = 7) were perfused with $^{18}O_2$-equilibrated blood for 10 min (Fig. 2 top). For LMF measurement, 120,000 blue microspheres were injected 5 min prior to termination of the experiments.

Early reperfusion

Baseline measurements were followed by 20 min no-flow ischemia (n=6). During reperfusion, $^{18}O_2$-equilibrated blood was used for 10 min (Fig. 2 middle). 120,000 blue microspheres were injected 5 min prior to ischemia, and 120,000 red microspheres were injected 5 min prior to termination of the experiments.

Late reperfusion

Baseline measurements and a 20 min no-flow period were followed by 30 min reperfusion. Thereafter, the hearts (n = 6) were perfused with $^{18}O_2$-equilibrated blood for an additional 10 min (Fig 2, bottom). LMF was measured as in the early reperfusion series.

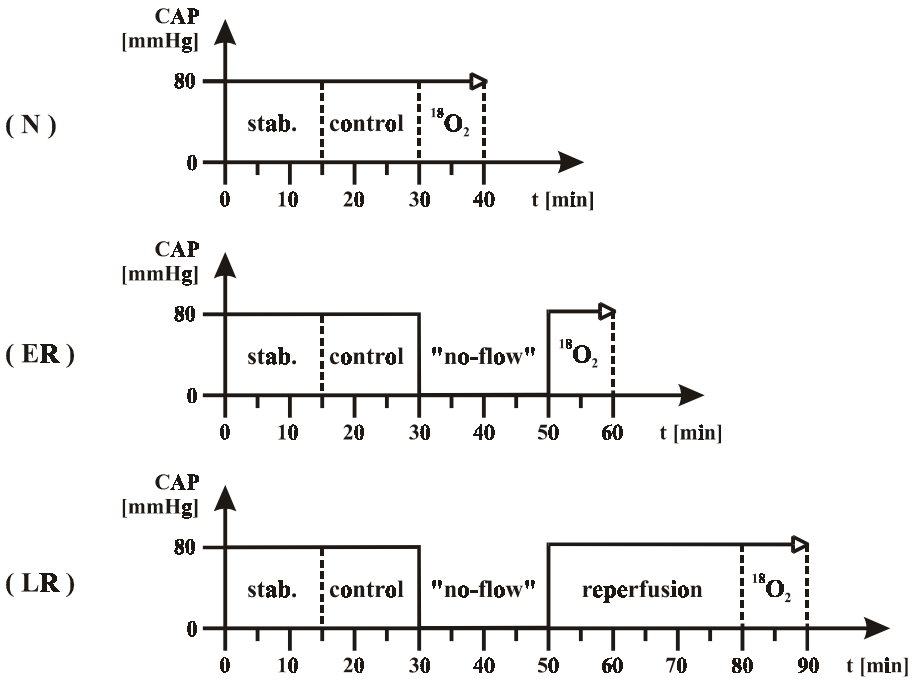

Fig. 2. *Protocols performed in the present study. (1) normoxia (top); (2) 10 min $^{18}O_2$-perfusion in early reperfusion following a 20 min no-flow ischemia (middle); (3) 10 min $^{18}O_2$-perfusion in late reperfusion following a 20 min no-flow ischemia and 40 min reperfusion (bottom).*

Myocardial tissue preparation

The frozen hearts were cut into basal, median and apical transversal slices. Samples of right ventricular free wall, septum and left ventricular free wall were dissected from the slices. The basal, median, and apical portions of the left ventricular free wall were further divided into subendocardial and subepicardial layers. A total of eight tissue samples were taken from each heart, with an average weight of 105 ± 15 mg.

Water extraction, $H_2{}^{18}O$ processing, and local blood flow

This procedure was described in detail previously (Schwanke *et al.* 1994, Schwanke *et al.* 1996). In brief, the water was extracted from the tissue samples during 2 h of lyophilization, after which 7.5 µl aliquots were taken and converted quantitatively to CO_2 using the guanidine hydrochloride technique (Dugan *et al.* 1985). The oxygen isotope ratio ($^{18}O/^{16}O$) within the CO_2 samples was determined using mass spectrometry (Finnigan, MAT 251, Bremen, DE). The oxygen isotope ratios were converted from the SMOW values [‰ SMOW] (Baertschi, 1976) to SI units and expressed as local $H_2{}^{18}O$ residue of cardiac tissue water per 1 g wet mass, thus representing a measure of local oxidative metabolism. Local myocardial flow was determined from the dried tissue samples by measuring the microsphere-associated colour intensity using spectrophotometry (Kowallik *et al.* 1991). Local blood flow data were normalized to 1 g wet mass. In all tissue samples, both local myocardial blood flow and the $H_2{}^{18}O$ residue were determined.

Calculations and statistics

Global myocardial oxygen consumption (MVO_2, $ml \cdot min^{-1} \cdot g^{-1}$) was calculated according to Fick's principle: $MVO_2 = CBF\ [ml \cdot min^{-1} \cdot g^{-1}] \cdot AVDO_2\ [mlO_2 \cdot (100\ ml)^{-1}]$. Both oxidative metabolism and local blood flow were normalized to the mean values of the individual experiment.

Hemodynamic data are presented as means ± S.D. The relations between (a) LMF and local oxidative metabolism and (b) LMF during baseline and reperfusion were determined by linear least-squares regression analysis using SYSTAT 5-0 software (SPSS Inc, Chicago/IL, US). A p-value < 0.05 was taken to indicate statistical significance.

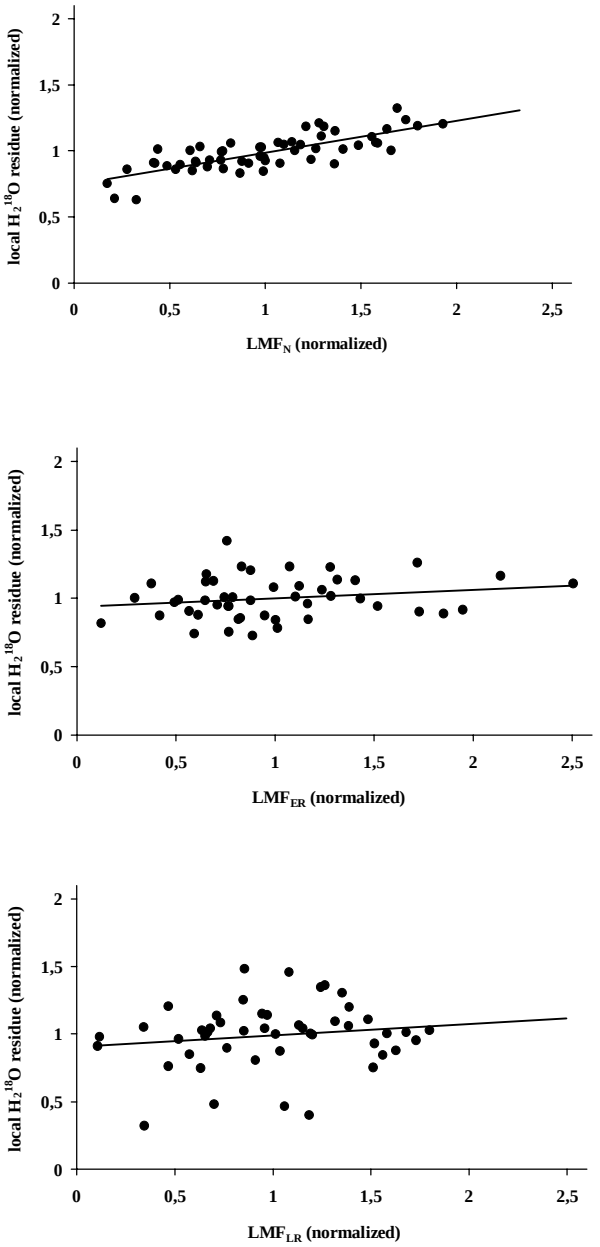

Fig. 3. *Linear regression between local myocardial blood flow (LMF) and local oxidative metabolism (local $H_2{}^{18}O$ residue after 10 min $^{18}O_2$ perfusion) within isolated, blood-perfused rabbit hearts.* ***Top:*** *Both variables correlated closely during normoxic control. The correlation coefficient (r) was equal to 0.77 ($p < 0.05$; n = 56 samples from 7 hearts;).* ***Middle:*** *During early reperfusion after 20 min total ischemia, LMF and local oxidative metabolism correlated less close: r = 0.20; n.s.; n = 48 from 6 hearts.* ***Bottom:*** *During late reperfusion after 20 min total ischemia, the correlation between LMF and local oxidative metabolism was completely lost: r = 0.15; n.s.; n = 48 from 6 hearts).*

Results

Table 1. Hemodynamics during normoxia

Heart rate [min^{-1}]	124 ± 16
Left ventricular pressure [mm Hg]	105 ± 9
Cardiac output [$ml \cdot min^{-1}$]	70 ± 11
Coronary flow [$ml \cdot min^{-1} \cdot g^{-1}$]	1.4 ± 0.5
MVO_2 [$ml \cdot min^{-1} \cdot g^{-1}$]	0.08 ± 0.01

Normoxia (N)

Hemodynamics and global myocardial oxygen consumption (MVO_2) are summarized in Table 1. In 56 tissue samples, local myocardial blood flow (LMF) ranged between 18 % and 193 % of the mean. Local oxidative metabolism varied between 63 % and 132 % of the mean. Linear regression analysis between both variables gave the equation: $H_2{}^{18}O = 0.24\ LMF + 0.75$ ($r=0.77$; $p < 0.001$; $n = 56$; Fig. 3 top).

Early reperfusion (ER)

Ventricular function was clearly depressed, coronary blood flow was increased to fivefold, and global MVO_2 was almost unchanged (Table 2).

Table 2. Hemodynamics during control (ctr) and after 20 min no-flow ischemia during early reperfusion (rep ≤ 10 min)

	ctr	**rep**
Heart rate [min^{-1}]	164 ± 26	86*± 13
Left ventricular pressure [mm Hg]	87 ± 7	51* ± 8
Cardiac output [$ml \cdot min^{-1}$]	45 ± 6	0* ± 0
Coronary flow [$ml \cdot min^{-1} \cdot g^{-1}$]	1.3 ± 0.1	6.5* ± 0.7
MVO_2 [$ml \cdot min^{-1} \cdot g^{-1}$]	0.08	0.06 ± 0.01

**p < 0.05 vs. ctr*

In 48 tissue samples, LMF ranged between 12 % and 250 % of the mean, and local oxidative metabolism ranged from 73 % to 142 %. The two variables did not correlate: $H_2{}^{18}O = 0.06\ LMF + 0.94$ ($r = 0.20$; n.s.; $n = 48$; Fig. 3 middle). Similarly, LMF during N and ER were only weakly correlated: $LMF_{ER} = 0.43 \cdot LMF_N + 0.57$ ($r = 0.24$; n.s.; $n = 48$; Fig. 4 top).

Late reperfusion (LR)

Ventricular function started to recover towards baseline values, while cardiac output was still showing signs of myocardial stunning. Coronary blood flow was only slightly lower than during baseline, whereas MVO_2 remained almost unchanged (Table 3).

In 48 tissue samples, LMF ranged between 11 % and 180 % of the mean, and local oxidative metabolism ranged from 32 % to 148 % of the mean. Linear regression analysis between both variables exhibited no correlation: $H_2{}^{18}O = 0.09\ LMF + 0.90$ ($r = 0.15$; n.s.; $n = 48$; Fig. 3 bottom). In contrast, LMF during N and LMF during LR correlated: $LMF_{LR} = 0.86 \cdot LMF_N + 0.17$ ($r = 0.87$; $p < 0.05$; $n = 48$; Fig. 4 bottom).

Table 3. Hemodynamics during control (ctr) and after 20 min no-flow ischemia during late reperfusion (rep ≥ 30 min)

	ctr	**rep**
Heart rate [min^{-1}]	183 ± 6	167 ± 20
Left ventricular pressure [mm Hg]	88 ± 5	80 ± 7
Cardiac output [$ml \cdot min^{-1}$]	41 ± 4	21* ± 10
Coronary flow [$ml \cdot min^{-1} \cdot g^{-1}$]	2.1 ± 0.8	1.5 ± 0.5
MVO_2 [$ml \cdot min^{-1} \cdot g^{-1}$]	0.08 ± 0.01	0.06 ± 0.01

**p < 0.05 vs. ctr*

Discussion

Using myocardial samples with an average mass of 105 ± 15 mg, we were close to the optimal sample size of about 80 mg to look at blood flow heterogeneity

(Bassingthwaighte *et al.* 1989), and by recovering 500 – 6000 spheres per sample, the methodological error is estimated to be only 3 - 9 % (Dole *et al.* 1982, Nose *et al.* 1985), i.e. considerably smaller than the differences found in local myocardial blood flow (LMF), which varied by a factor of nearly 10.

The heterogeneity in LMF found in the present study was almost independent of the actual protocol and agrees well with data in the literature (King and Bassingthwaighte 1989, Bassingthwaighte *et al.* 1990, Deussen *et al.* 1996, Sonntag *et al.* 1996) and our recent study on buffer-perfused hearts (Schwanke *et al.* 2000).

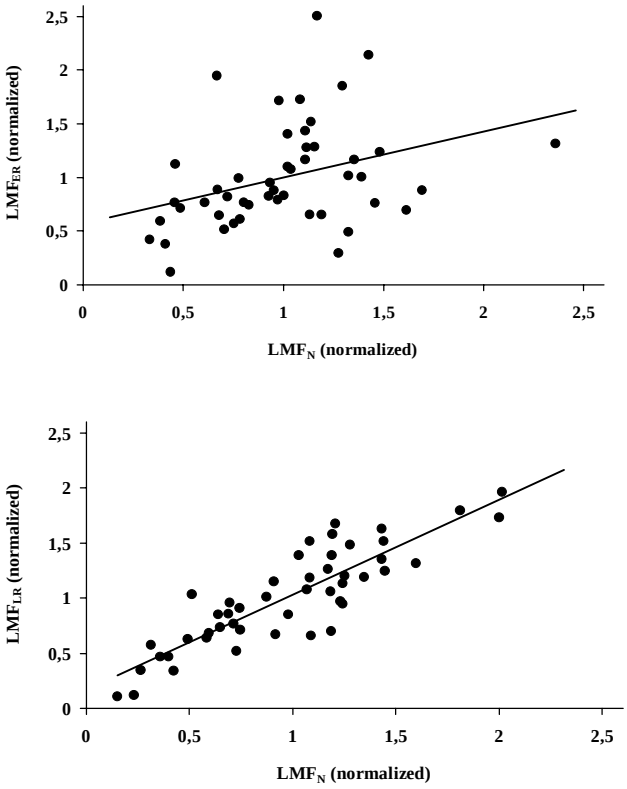

Fig. 4. *Linear regression between local myocardial blood flow (LMF) during normoxia and during reperfusion.* ***Top:*** *LMF during normoxic control and during early reperfusion after 20 min total ischemia were not closely correlated, indicating that the flow pattern during early reperfusion had changed compared with normoxic control:* $LMF_{ER} = 0.43\ LMF_N + 0.57$ *(r = 0.24; n.s.; n = 48).* ***Bottom:*** *LMF during normoxic control and during late reperfusion closely correlated indicating that the LMF patterns during normoxic control and during late reperfusion were similar:* $LMF_{LR} = 0.86\ LMF_N + 0.17$ *(r = 0.87; p < 0.05; n = 48)*

The spatial blood flow pattern observed at baseline changed during early reperfusion, i.e. during reactive hyperemia. Yet, after reactive hyperemia had subsided and blood flow was slightly below baseline during late reperfusion, the postischemic flow pattern was almost identical with the baseline pattern, the slope between both LMFs being only somewhat below the line of identity (Fig. 4). We have thus confirmed earlier findings (Schulz *et al.* 1991) that the dysfunction in stunned myocardium cannot be attributed to an insufficient blood flow *per se*.

Most techniques measure respiratory activity, and hence local oxygen consumption, only indirectly using labelled substrates (Breull *et al.* 1984, Groeneveld and Visser, 1993, Kuschinsky *et al.* 1993, Müller *et al.* 1993, Deussen and Bassingthwaighte 1996, Deussen 1997). These techniques have a major drawback (Bassingthwaighte and Goresky 1984, Deussen and Bassingthwaighte 1996), because myocardial substrate metabolism varies greatly with the physiological state. Such changes in substrate utilization occur in particular in the stunned myocardium (Renstrom and Liedtke 1995), when fatty acid oxidation recovers quickly during reperfusion and dominates as a source of oxygen consumption (Lerch 1995, Kantor *et al.* 1999, van de Velde *et al.* 2000). Our technique directly determines the transfer of oxygen to oxidation water and thus provides a direct measure of local oxidative phosphorylation activity. Hence, although the blood used in the present study contained both glucose and fatty acids, any protocol-dependent shift in substrate utilization during reperfusion will probably not have affected our results concerning oxidative metabolism.

The most direct measure of local aerobic metabolism is the local production rate of oxidation water. This rate is difficult to measure directly, and therefore the local residue of oxidation water must serve as a reasonable index of the local production rate. Since the local ^{18}O water tissue residue depends both on intracellular production and simultaneous washout from the tissue, a mathematical model analysis (Deussen and Bassingthwaighte 1996) is necessary to calculate the local MVO_2. Such analysis, however, seemed difficult for the present study, since the permeability surface area product in stunned myocardium is not known. Therefore, the ^{18}O water residue served as an estimate of local MVO_2 in the present study.

The correlation between local flow and oxidative metabolism at baseline was largely lost during early

reperfusion (r = 0.20) and even worse at late reperfusion (r = 0.15). Because the flow heterogeneity during late reperfusion was similar to that during normoxia it appears that it is not the loss in flow heterogeneity but a loss in metabolic heterogeneity, which is associated with myocardial stunning, reflecting a mismatch between local flow and local metabolism.

We hypothesize that local metabolism reflects local myocardial function. Unfortunately, we can only speculate on contractile function on the microregional level (~ 80 mg) as no techniques to measure function at that resolution are available. Since low-flow / low-metabolism areas do not become necrotic with a blood flow as low as 0.2 $ml \cdot min^{-1} \cdot g^{-1}$, their local function is also expected to be low. In turn, high-flow in high-metabolism areas appears not to reflect luxury perfusion (Bassingthwaighte and Goresky 1984, King and Bassingthwaighte 1989, Deussen *et al.* 1996), and therefore their function is expected to be high.

We propose that the difference in local $H_2{}^{18}O$ residue between low-flow and high-flow areas reflects increased reperfusion/reoxygenation injury in post-ischemic high-flow areas. If so, local function in high-flow areas is very likely also more compromised than that in low-flow areas secondary, at least in part, to the decreased efficiency as a result of fatty acid oxidation at the expense of glucose oxidation. A very similar finding was reported using a different experimental approach: high-flow areas in the myocardium of baboons were more susceptible to undergo necrosis during ischemia (Ghaleh *et al.* 1996).

Critique of methods

One factor might have introduced an error into our results that is related to the baseline measures of the three series. We acknowledge the differences between the series and attribute them to biological variations. On the other hand, the ventricular function does not differ considerably, if expressed in terms of the rate pressure product (13,020 *vs.* 14,268 *vs.* 16,104 mm Hg/min) which nicely fits with the global MVO_2 that was almost identical in the three series. In turn, we exclude a learning curve effect, since both the protocol and the experimental model is being used in our laborartory for a long time (Schipke *et al.* 1996, Schwanke *et al.* 2000).

In conclusion

We propose that myocardial stunning is due to the functional failure of high-flow areas and the inability of low-flow areas to compensate for the functional loss. Such mismatch between local flow and metabolism may contribute to myocardial stunning.

Acknowledgements

Dr. U. Schwanke was supported by a fellowship from the German Cardiac Society. This study was supported by grants from the German Research Foundation (DFG; He 1320/10-1 and Schi 201/11-1).

References

BAERTSCHI P: Absolute ^{18}O-content of standard mean ocean water. *Earth and Planetary Science Letters* **31:** 341-344, 1976.

BASSINGTHWAIGHTE JB, GORESKY CA: Analysis of capillary-tissue exchange. In*: Handbook of Physiology, Section 2: The Cardiovascular System*. EM RENKIN, CC MICHEL (eds), Bethesda, Maryland, American Physiological Society, 1984, pp 549-622.

BASSINGTHWAIGHTE JB, KING RB, ROGER SA: Fractal nature of regional myocardial blood flow heterogeneity. *Circ Res* **65:** 578-590, 1989.

BASSINGTHWAIGHTE JB, MALONE MA, MOFFETT TC, KING RB, CHAN IS, LINK JM, KROHN KA: Molecular and particulate depositions for regional myocardial flows in sheep. *Circ Res* **66:** 1328-1344, 1990.

BOLLI R: Mechanism of myocardial "stunning". *Circulation* **82:** 723-738, 1990.

BOLLI R, PATEL BS, HARTLEY CJ, THRONBY JI, JEROUDI MO, ROBERTS R: Nonuniform transmural recovery of contractile function in stunned myocardium. *Am J Physiol* **257:** H375-H385, 1989.

BREULL W, KÜSTERS W, NABERSCHNIG R: Regional glucose consumption and oxygen supply in the isolated perfused working rat heart. *Adv Exp Med Biol* **180:** 721-730, 1984.

DEUSSEN A: Local myocardial glucose uptake is proportional to, but not dependent on blood flow. Pfluegers Arch **433:** 488-469, 1997.

DEUSSEN A, BASSINGTHWAIGHTE JB: Modeling [^{15}O] oxygen tracer data for estimating oxygen consumption. *Am J Physiol* **270**, H1115-H1130, 1996.

DEUSSEN A, FLESCHE CW, LAUER T, SONNTAG M, SCHRADER J: Spatial heterogeneity of blood flow in the dog heart. II: Temporal stability in response to adrenergic stimulation. *Eur J Physiol* **432:** 451-461, 1996.

DOLE WP, JACKSON DL, ROSENBLATT JI, THOMPSON WL: Relative error and variability in blood flow measurements with radiolabeled microspheres. *Am J Physiol* **243:** H371-H378, 1982.

DUGAN JP, BORTHWICK J, HARMON RS, GAGNIER MA, GLAHN JE, KINSEL EP, MACLEOD S, VIGLINO JA, HESS JW: Guanidine hydrochloride method for determination of water oxygen isotope ratios and the oxygen-18 fractionation between carbon dioxide and water at 25 °C. *Anal Chem* **57:** 1734-1736, 1985.

GHALEH B, SHEN YT, VATNER SF: Spatial heterogeneity of myocardial blood flow presages salvage versus necrosis with coronary artery reperfusion in conscious baboons. *Circulation* **94:** 2210-2215, 1996.

GROENEVELD ABJ, VISSER FC: Correlation of heterogeneous blood flow and fatty acid uptake in the normal dog heart. *Basic Res Cardiol* **88:** 223-232, 1993.

HEYNDRICKX GR, MILLARD RW, MCRITCHIE RJ, MAROKO PR, VATNER SF: Regional myocardial functional and electrophysiological alterations after brief coronary artery occlusion in conscious dogs. *J Clin Invest* **56:** 978-985, 1975.

KANTOR PF, DYCK JR, LOPASCHUK GD: Fatty acid oxidation in the reperfused ischemic heart. *Am J Med Sci* **318:** 3-14, 1999.

KING RB, BASSINGTHWAIGHTE JB: Temporal fluctuations in regional myocardial flows. *Pfluegers Arch* **413:** 336-342, 1989.

KING RB, BASSINGTHWAIGHTE JB, HALES JRS, ROWELL LB: Stability of heterogeneity of myocardial blood flow in normal awake baboons. *Circ Res* **57:** 285-295, 1985.

KOWALLIK P, SCHULZ R, GUTH BD, SCHADE A, PAFFHAUSEN W, GROSS R, HEUSCH G: Measurement of regional myocardial blood flow with multiple colored microspheres. *Circulation* **83:** 974-982, 1991.

KUSCHINSKY W, BÜNGER R, SCHRÖCK H, MALLET RT, SOKOLOFF L: Local glucose utilization and local blood flow in hearts of awake rats. *Basic Res Cardiol* **88:** 233-249, 1993.

LERCH R: Myocardial stunning: the role of oxidative substrate metabolism. *Basic Res Cardiol* **90:** 276-278, 1995.

MÜLLER FU, HUNNEMAN DH, KAHLES R, HELLIGE G: Investigation of cardiac metabolism using stable isotopes and mass spectrometry. *Basic Res Cardiol* **88:** 272-281, 1993.

NOSE Y, NAKAMURA T, NAKAMURA M: The microsphere method facilitates statistical assessment of regional blood flow. *Basic Res Cardiol* **80:** 417-429, 1985.

RENSTROM B, LIEDTKE AJ: Adjustments in competitive substrate utilization in stunned myocardium during early reperfusion. *Basic Res Cardiol* **90:** 279-281, 1995.

SCHIPKE JD, KORBMACHER B, DORCZEWSKI A, SELCAN G, SUNDERDIEK U, ARNOLD G: Hemodynamic and energetic properties of stunned myocardium in rabbit hearts. *Heart* **75:** 55-61, 1996.

SCHULZ R, JANSSEN F, GUTH BD, HEUSCH G: Effect of coronary hyperperfusion on regional myocardial function and oxygen consumption of stunned myocardium in pigs. *Basic Res Cardiol* **86:** 534-543, 1991.

SCHWANKE U, STRAUSS H, BERTRAM HG, ARNOLD G, SCHIPKE JD: A new technique for measurement of myocardial O_2-utilization. *Isotopenpraxis Environ Health Stud* **30:** 133-139, 1994.

SCHWANKE U, STRAUSS H, ARNOLD G, SCHIPKE JD: Analysis of respiratory water - a new method for evaluation of myocardial energy metabolism. *J Appl Physiol* **81:** 2115-2122, 1996.

SCHWANKE U, DEUSSEN A, HEUSCH G, SCHIPKE JD: Heterogeneity of local myocardial flow and oxidative metabolism. *Am J Physiol* **279:** 1029-1035, 2000.

SONNTAG M, DEUSSEN A, SCHULTZ J, LONCAR R, HORT W, SCHRADER J: Spatial heterogeneity of blood flow in the dog heart. I. Glukose uptake, free adenosine and oxidative/ glycolytic activity. *Eur J Physiol* **432:** 439-450, 1996.

THAULOW E, GUTH BD, HEUSCH G, GILPIN E, SCHULZ R, KRÖGER K, ROSS J Jr: Characteristics of regional myocardial stunning after exercise in dogs with chronic coronary stenosis. *Am J Physiol* **257:** H113-H119, 1989.

VAN DE VELDE M, DEWOLFF M, LEATHER HA, WOUTERS PF: Effects of lipids on the functional and metabolic recovery from global myocardial stunning in isolated rabbit hearts. *Cardiovasc Res* **48:** 129-137, 2000.

Reprint requests

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